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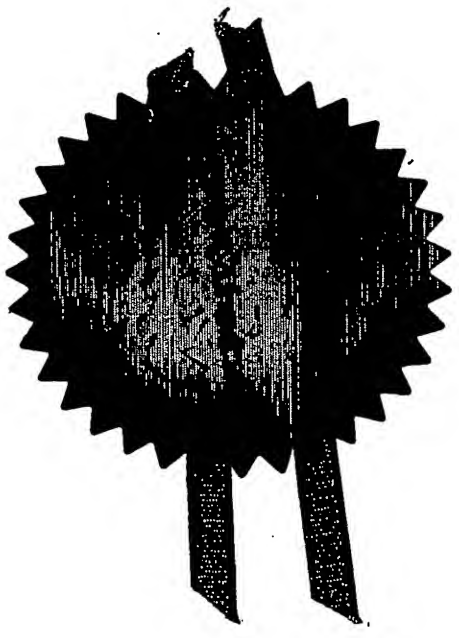

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THE UNIVERSITY OF READING
PO Box 217
Whiteknights
Reading
Berkshire RG6 6AH

ASTRAZENECA AB
S-151 85
Sodertalje
Sweden

Patents ADP number *(if you know it)*

764300000 2

78226480 03

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GB

SE

4. Title of the invention

Composite Nanoparticles

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

MEWBURN ELLIS
York House
23 Kingsway
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COMPOSITE NANOPARTICLES

FIELD OF THE INVENTION

The present invention relates to nanoparticles with a porous surface, methods of making such nanoparticles and their uses in measuring partition coefficients of molecules and in encapsulation of catalytically active species, such as biologically active species.

BACKGROUND TO THE INVENTION

The term "nanoparticles" is used to describe particles with dimensions on a nanometre scale. Generally these particles may range in size from around 1 nm up to 1 μm typically having dimensions of between 1 nm and a few hundreds of nanometres. Due to their small size, nanoparticles have a very large surface area to volume ratio. This feature explains the reason why many of the uses of nanoparticles are in processes requiring a maximised surface area with the lowest possible volume such as many heterogeneous catalysis reactions.

Nanoparticles can vary in their internal structure. The simplest particles consist of just a single material whilst more complex particles may have a core region with one or more different layers, formed from different materials, arranged around it.

There are a number of methods of making nanoparticles ranging from simple grinding and milling

techniques, through deposition from a microemulsion and polymerisation of emulsions to electric arc vaporisation of a material. The method used depends upon the complexity of the particle which is required e.g. the number of layers of different material in the particle, how the different layers interact and other well defined parameters.

Whilst nanoparticles made from a single material are the simplest to manufacture, by simple milling techniques, particles with an outer coating on them are nevertheless widely used in order to protect the inner core of the particle from chemical or physical degradation.

Methods of making nanoparticles with a variety of different core materials and a surface layer are well known. US 6,548,264 discloses a range of particles with a silica coating on the outside and methods of making them using microemulsions.

PCT/GB2003/000029 (Reading University) also describes a method of making silica particles having a magnetic core. Methods of making nanoparticles of other coated materials, such as alumina, titania or zirconia by sol-gel technology or related techniques are known.

SUMMARY OF THE INVENTION

The present invention makes use of the porosity of the surface layer of nanoparticles, in the application of

such particles to new uses. The porosity can be controlled in the manufacture of such particles. The manufacturing method also permits the introduction of molecules of interest in the core of the particle, inside the porous coating or layer, the porosity of the particle providing access to the entrapped molecule.

In one aspect of the present invention nanoparticles are prepared and used in a process for determination of the partition coefficient of a molecule in a solvent system consisting of two immiscible solvents. The partition coefficient of a molecule is dependent upon the solvent system in which it is measured and gives a numerical assessment of how the molecule is distributed between the two solvents at equilibrium. This has one particular use in pharmaceutical drug development.

The present state of the art for measuring partition coefficient values is described in "Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies" (B. Testa, H. van de Waterbeemd, G. Folkers, R. Guy (editors), Verlag Helvetica Chimica Acta, Zurich, 2001). In the most generally applied measurement, a test molecule is dissolved to a known concentration in a first solvent e.g. water. A known amount of this solution is then added to a known amount of a second solvent e.g. n-octanol and the two phases are well mixed. The system is then allowed to reach concentration equilibrium. Finally

the phase containing the first solvent is separated out and the concentration of the test molecule in this solution is measured. From this value, a partition coefficient (P) can be determined.

A number of problems arise with this method of determining the partition coefficient. When the two immiscible phases are mixed, it is necessary to allow them to equilibrate for a long time to reach concentration equilibrium. This is due to the relatively low contact area between the two phases. Also, the separation of the two phases requires a visual assessment of the position of the inter-phase boundary. If a molecule is highly soluble in one phase, it may be necessary to use a very small amount of that solvent which makes assessment of the position of the inter-phase boundary difficult. In addition, the fact that the inter-phase boundary must be visible for the phases to be separated requires relatively large volumes of solvents to be used. This generates large volumes of hazardous waste and adds to the cost of the procedure.

The combination of these problems makes this process a costly and time-consuming one, especially when performed on an industrial scale.

The present invention in one aspect seeks to overcome the above problems by using nanoparticles in a method of measuring the partition coefficient of a test molecule.

In a first aspect therefore the invention provides a method of determining the partition coefficient of a chemical compound between two solvents in a mixture containing a first solvent and a body of nanoparticles, wherein a second solvent is absorbed in the pores of nanoparticles. A body of nanoparticles having the solvent absorbed in them can provide a predetermined quantity of the solvent, which can be very small, allowing determination of extreme partition coefficients. The nanoparticles can be easily separated from the first solvent, for example when the nanoparticles have a magnetic core permitting magnetic separation. Other methods of separation are available such as centrifugation, or changing the dielectric constant of the system, e.g. by addition of another solvent, to cause precipitation of the nanoparticles; the nanoparticles employed in this aspect of the invention therefore may consist only of the porous material.

It has been shown that the use of nanoparticles in the measuring of partition coefficients does not affect the results i.e. the nanoparticles do not significantly influence the value of the partition coefficient obtained. The procedure can be quick, since the equilibrium distribution of the measured compound is obtained rapidly, and economical since the easy and effective separation of the nanoparticles allows small quantities of one or both solvents to be used.

It is desirable, for accurate determination of the partition coefficient, that the first solvent phase is pre-saturated with the second solvent phase and vice versa. Therefore it is not necessary that the two solvents are mutually wholly insoluble; what is important is that they form two immiscible phases.

The present invention also consists in compositions containing nanoparticles which are useful in this method of determining partition coefficients. There is provided a mixture of nanoparticles having a porous outer coating, and a first solvent, wherein a second solvent is absorbed into the porous coating of the nanoparticles and wherein said first and second solvents are immiscible. Such a composition is stable e.g. as a colloidal dispersion of the particles in the first solvent, has a long shelf life and permits easy and accurate dispensing of a predetermined quantity of the second solvent. In this composition, preferably the second solvent phase is wholly absorbed in the nanoparticles, i.e. does not appear freely outside the nanoparticles.

The amount of the nanoparticles containing the second solvent per unit volume of first solvent (i.e. also per unit volume of the total composition) can thus be predetermined, i.e. known, and fixed for a particular composition. The nanoparticles can be uniformly distributed in the first solvent, as a colloidal solution. Thus the ratio of the volumes of the two

solvents is accurately predetermined. Volumetric dispensing of a quantity of the composition can therefore be performed, providing in an easy manner any desired volume of the two solvents in a predetermined ratio. High accuracy can be achieved.

The composition is preferably stored in a sealed container, to prevent evaporation.

The first solvent in which the nanoparticles are suspended is preferably aqueous e.g. water or an aqueous solution or a water-containing phase.

The second solvent which is absorbed into the porous outer coating of the nanoparticles may for example be a water-immiscible solvent, e.g. n-octanol, cyclohexane, alkane (C_6-C_{10}), chloroform, propylene glycol dipelargonate (PGDP), 1,2-dichloroethane, olive oil, benzene, toluene, nitrobenzene, chlorobenzene, tetrachloromethane, oleyl alcohol, 4-methylpentan-2-ol, pentan-1-ol, pentan-2-ol, isobutanol, butan-1-ol, 2-methylbutan-2-ol, butan-2-ol, butan-2-one, diethyl ether, isoamyl acetate, ethyl acetate, etc.

Both solvents are preferably free of any biologically active compound, particularly any pharmaceutically active compound.

An alternative form of composition provided by the invention, also suitable for accurate dispensing of a predetermined amount of a solvent (i.e. the solvent called the second solvent above) in a form convenient for

a quantitative analytical procedure such as the partition coefficient determination herein described, is a composition comprising nanoparticles each having a porous surface and the solvent adsorbed in the pores of the nanoparticles in a predetermined amount per unit weight of the composition. In this composition, preferably there is no free solvent (i.e. it is all absorbed in the nanoparticles), so that the composition is effectively a particulate solid and is dispensable by weighing (gravimetrically). The amount of the solvent is thus predetermined for unit weight of the composition. This composition also is preferably stored in a sealed container, to prevent evaporation of the solvent. The solvent may be substantially free of any solute, e.g. free of any biologically active compound. This composition can be accurately mixed with a desired quantity of another solvent (called first solvent above), to obtain a composition of two solvents as described above; this may be done for example by user, immediately prior to use.

The invention in a second aspect arises from the finding that a catalytically active species, especially a biologically active species, especially a biological catalyst, can be entrapped in the cores of porous nanoparticles in a state in which its catalytic activity is maintained and in which substrate molecules can access it via the pores of the particle for catalytic reaction

to occur. This is due to the porous coating having a pore size smaller than the size of the biologically active species. One advantage is that the bioactive species may be entrapped without chemical bonding, so that it is essentially in its free state of optimum nature. Its activity may therefore not be impaired or altered, in contrast with known techniques in which molecules are chemically bonded to a support.

By control of particle size, and in particular core size, it is possible to provide a body of nanoparticles having a known, reproducible quantity of the entrapped species. The core size may be such that only one molecule of the bioactive species is present; in this case, in a population of the nanoparticles, some may contain no catalytic molecule and some may contain more than one. It is possible therefore to provide a population or assembly of nanoparticles containing on average not more than one molecule of the catalytically active species per particle.

One advantage of this entrapment is to reduce aggregation or agglomerization of the bioactive species (reduce the formation of dimer, trimer, tetramer and so on) by means of the coating, which reduces the extent of deactivation.

The nanoparticles containing catalytically active species in this aspect of the invention can be employed in many applications, e.g. enzymatic reactions and other

catalytic reactions, assay methods (e.g. by binding of target molecules to the entrapped species such as antigen-antibody reactions; protein-drug binding; bioreceptor-antigen binding, oligonucleotide recognition; biotin-streptavidin reactions), and as biosensors etc.. An important advantage is to trap a free form of bulky bioactive species inside the core of the nanoparticle with a porous coating of tailored size. This prevents leaching of the trapped species to solution through the coating. On the other hand the pore size of the coating allows the exchange of small molecules (smaller than the pore size), permitting access to the trapped molecules freely. Separation can be therefore achieved using trapped core magnet(s) or by other means. As a result, the porous coating of the composite nanoparticles can be regarded as a 'nano-membrane' for molecular recognition and separation.

In addition, the nanoparticles of the present invention encapsulating catalytically active species can allow catalysis to be performed on a small scale and allowing simple separation of products from a heterogeneous catalyst.

BRIEF DESCRIPTION OF THE FIGURES

Fig 1 shows the correlation between logD results achieved by measurement using nanoparticles and literature values.

Fig 2 shows the correlation between logD results achieved by measurement using nanoparticles and values obtained using the prior art method.

Fig 3 shows the magnetic field response of the particles obtained using the method of example 1.

Fig 4 shows a transmission electron microscopy (TEM) micrograph of the silica coated particles produced by the method of example 1.

Fig 5 shows an x-ray diffraction (XRD) pattern of the silica-coated Fe_3O_4 nanoparticles obtained in example 1 recorded using a wavelength of 1.54056 nm.

Fig 6 shows a thermogravimetric (TG) analysis of the silica coated Fe_3O_4 nanoparticles obtained in example 1.

Fig 7 shows a thermogravimetric (TG) analysis of the silica coated Fe_3O_4 nanoparticles in which the silica coating has been saturated with n-octanol.

Fig 8 shows two infra red (IR) spectra: a) is of the silica coated Fe_3O_4 nanoparticles; b) is of the silica coated Fe_3O_4 nanoparticles which have been treated with chlorotrimethyl silane (CTMS).

Fig 9 shows an XRD pattern of the Fe_2CoO_4 nanoparticles obtained in example 5.

Fig 10 shows a UV-visible spectrum of a penicillin V solution in the presence of β-lactamase I.

Fig 11 shows a UV-visible spectrum of a penicillin V solution in the presence of a micellar solution of β-lactamase I.

Fig 12 shows a UV-visible spectrum of a penicillin V solution in the presence of β-lactamase I which is encapsulated inside a porous silica coating.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Formation and properties of porous-coated nanoparticles.

As mentioned above solid nanoparticles having a core surrounded by a porous coating can be made by a method which includes the steps of:

(a) forming, in a liquid medium, colloidal particles containing a core species and colloidally stabilized by organic stabiliser(s) or stabilized as micellar aggregates (e.g. stabilised water droplets embraced by surfactant molecules), and

(b) forming a porous coating around the colloidal particles by hydrolyzing a precursor compound in the region of the interface between the colloidal particle or micellar particle and the liquid medium.

Preferably the nanoparticles are aged, e.g. for an hour to weeks, preferably 2-5 days, before removal from the colloidal system, in order to establish the porous coating to the desired thickness.

The porous coating formed around the core of the particle may be formed from a range of porous materials such as alumina, silica, titania, zirconia or carbon. Preferably the porous coating is formed from silica by

hydrolysis of a silicon-containing compound at the interface region of the colloidal suspension.

The compound which is hydrolyzed may be an alkoxy silane compound, i.e. a compound containing at least one Si-OR linkage, where R is alkyl of preferably 1 - 8 carbon atoms, more preferably 1 - 4 carbon atoms, such as tetraethyl ortho silane (TEOS, $Si(OC_2H_5)_4$); and chloro-, bromo-, hydro- and metallo- silanes, (containing Si-Cl, Si-Br, Si-H or Si-M bonds where hydrolysis occurs). Alternatively, the compound which is hydrolysed may be an analogous alkoxy, halo, hydro compound of titanium, aluminium or zirconium (or an intermetallic compound) such as titanium isopropoxide or titanium tetrachloride.

After hydrolysis of the above compound(s) the described aging process allows cross condensation of the -OH species, forming a three-dimensional gel (with e.g. Si-O-Si or Ti-O-Ti linkage) embracing the particle therein.

For the formation of a carbon coating, the colloidal particles formed in step (a) are separated from the colloidal suspension and are pyrolysed so that the organic surfactant coating around the particles decomposes to form a porous carbon outer coating around the nanoparticle core. Other carbon precursor(s) such as polyvinyl alcohol, phenol/polyphenols, polysaccarides, etc could be used for the porous carbon formation.

In a preferred form of the method in step (a) the colloidal particles are made by forming an emulsion having dispersed phase droplets or micelles stabilized by the surfactant and containing a dissolved compound of a core material and causing the core species to precipitate thereby forming the colloidal particle inside the micelles. The precipitation may be caused by addition of alkali or ammonia.

Preferred surfactants used for stabilising the colloidal particles include cetyltrimethylammonium bromide (CTAB), oleic acid, polyvinylpyrrolidone (PVP), non-ionic surfactants such as AOT, TX100, etc.

The porous material may have at its surface functional groups, e.g. OH groups, for the chemical (e.g. covalent) attachment of other species, such as biochemical or biological species (e.g. peptides, markers, cognate binding partner, solubilizers) or attachment of the nanoparticle to a substrate with or without the use of linker molecules. Immobilisation of charged species on charged surface at defined pH by electrostatic interactions is also included.

A plurality of metal-containing species of different metals may be included in the colloidal particles, and thus in the core of the nanoparticles produced. Typically such a metal-containing species is selected from metal, alloy, metal oxide, metal hydroxide and metal carbide. Preferably the metal-containing species is

ferromagnetic (enabling magnetic separation of the nanoparticles) or super-paramagnetic, or single domain magnetic nanoparticles are employed. Magnetic materials which may be included in the core of the nanoparticles include magnetite (Fe_3O_4), maghemite (γFe_3O_4), greigite (Fe_3S_4) and Fe_2CoO_4.

The cores of the nanoparticles may alternatively or additionally comprise a catalytically or biologically active species. Preferred biologically active species include enzymes and proteins. Examples are lactase, metallothionin, cytochrome (such as cytochrome b, cytochrome c or cytochrome P450), blood albumin(s), carboxylesterases, kinase, short nucleic acid oligomers, antibody species and enzyme indicators in blood/liver tissues. referred catalytic species include inorganic catalyst compounds (e.g. formed by "ship in a bottle chemistry") such as heteropolyacids, metallothioleins, corands, coraplexes, spherands, spheraplexes, cavitands, host-guest catalysts, and intercalated catalysts, etc.

Where the cores of the nanoparticles include a catalytically or biologically active species, it is preferred that the porous coating has a pore size smaller than the catalytically or biologically active species so that the active species is retained inside the coating of the nanoparticle.

Furthermore, it is preferred that the porous coating of the nanoparticle has a pore size which is large enough

to allow small molecules to pass through. In particular, it is preferred that where a catalytically active species is encapsulated in the core of the nanoparticle, the pore size of the porous outer coating is larger than the size of both the reactant and the product of the catalytic reaction. In this case, a reactant molecule may pass through the porous coating of the nanoparticle, interact with the catalytically or biologically active species retained inside the nanoparticle and products from the interaction may pass out through the porous coating.

The nanoparticles preferably have an average diameter in the range 1 nm to 1 μm, more preferably 1 to 100 nm. The porous coating may have any desired thickness, but preferably has an average thickness in the range 1 to 100 nm, preferably 1 to 50 nm. Where the core of the nanoparticle comprises a catalytically or biologically active species, the diameter of the core may be between 1 and 10 nm and is preferably between 1 and 5 nm.

Use of porous-coated nanoparticles in the measurement of the partition coefficient of a test molecule.

The present invention provides a method of attaining partition of a test molecule between two immiscible solvents through the use of porous nanoparticles. The method comprises the step of mixing the test molecule with a first solvent of a colloidal suspension comprising

nanoparticles with a porous outer coating wherein a second solvent is absorbed into the porous outer coating, the nanoparticles being suspended in the first solvent which is immiscible with the second solvent. The test molecule dissolves partially in the second solvent, and is retained in the porous outer coating of the nanoparticles, and partially in the first solvent.

In order to optimise the speed with which partition of a test molecule is achieved, it is desirable to maximise the contact area between the two immiscible solvents. This can be achieved by forming particles with a high ratio of surface area to volume, i.e. the smaller the diameter of the particle, the faster partition will be achieved. In addition, the porous coating on the nanoparticles should absorb as much solvent as possible in order to speed up the partitioning of the test molecule. As such, small particles with large pore volumes are preferred. If the features of the porous coating which favour a fast partitioning of the test molecule are optimised, partitioning times in the region of 1 - 10 minutes or less may be achieved using nanoparticles of the present invention. This represents a significant improvement over the partitioning times achieved in the prior art.

In this method the compositions comprising solvents and nanoparticles described above may be employed.

The present method of attaining partition of a test molecule between two immiscible solvents may be used to determine the value of the partition coefficient for the test molecule.

The following terms will be used in the discussion of partition coefficients:

logP is the standard value quoted for the partition coefficient of a test molecule where P = $[C]_{organic}/[C]_{aqueous}$. Unless otherwise specified, these values are recorded for an octanol-water biphasic system using the molecule in its electronically neutral form.

logD is the most commonly used value in this specification. This refers to the partition coefficient of a test molecule at a specified pH value. In calculating these values the following relation is used for D:

$$D_{pH} = f_N \times P_N + f_I \times P_I$$

where f_N and f_I are the molar fractions of the neutral and ionised forms of the test molecule respectively and P_N and P_I are the P values for the neutral and ionised forms of the test molecule respectively.

One method of measuring the partition coefficient (either logP or logD) value for a test molecule comprises the steps of:

a) providing a composition of nanoparticles, with a porous surface and a first solvent wherein a second solvent has been absorbed into the porous surface, and

said first solvent is immiscible with said second solvent;

b) incorporating a molecule to be tested in a composition of step a); and

c) separating the product of step b) into two components, the first comprising the nanoparticles and the second comprising the first solvent; and

d) the amount of the molecule to be tested which remains in the first solvent may be determined to enable calculation of the partition coefficient.

Step c) of this method may be achieved by e.g. filtration or centrifugation of the product of step b) to separate the mixture into the two components comprising the nanoparticles and the supernatant solution, or by other separation methods mentioned above. In the case where the core of the nanoparticle contains a magnetic material, a magnetic field may alternatively be used to perform step c) of the above method. In this case, a magnetic field applied to the product of step b) can be made to precipitate the nanoparticles from the reaction mixture.

Step d) of the method of measuring the partition coefficient may be achieved by any analytical technique through which the concentration of the test molecule in a solution can be determined. These techniques may include nuclear magnetic resonance (NMR), titration, UV-visible spectroscopy, fluorescence, phosphorescence, high-

performance liquid chromatography (HPLC), gas chromatography(GC), mass spectroscopy(MS), GC-MS, gravimetric, surface plasma and electro-analytic techniques. Preferably the technique used in step d) does not require further processing of the supernatant solution and may be performed without removal of a sample from the reaction vessel. In a most preferred embodiment, the technique used in step d) is UV-visible spectroscopy.

In the preferred case where UV-visible spectroscopy is used to determine the concentration of the test molecule in the supernatant solution, the following equation may be used to calculate logD:

$$\log D = \log \{[(A1-A2)/A2] \times V_1/V_2\}$$

where A1 = UV-visible absorption of the test molecule in the supernatant phase before partitioning.

A2 = UV-visible absorption of the test molecule in the supernatant phase after partitioning.

V_1 = Volume of first solvent (with which the nanoparticles are mixed).

V_2 = Volume of second solvent (absorbed into the porous outer coating of the nanoparticles).

Correlation of the logD values measured by the method of the present invention and those measured by the prior art method is demonstrated by figures 1 and 2.

The selection of the ratio of second solvent (absorbed into the porous outer coating of the

nanoparticle) to the first solvent is made depending upon the approximate solubility of the test molecule in the two solvents (which if not known previously may be estimated or may be arrived at by experiment). In the present invention, the ratio of first solvent to second solvent may be between 3000:1 and 1:1. Typically the ratio of first solvent to second solvent is greater than 50:1 and preferably 100:1 or greater.

This method of measuring the partition coefficient of a test molecule has a number of distinct advantages over the prior art methods.

First, the partition of the test molecule is achieved faster than in the prior art for the reasons already discussed.

Secondly, in the case where the nanoparticle core contains a magnetic material, the mixture used to measure the partition coefficient can be easily separated into a nanoparticle component and a supernatant solution by application of a magnetic field to the solution. Typically separation of these two components of the mixture can be achieved in a matter of seconds.

Thirdly, as one of the solvents is absorbed into the porous coating of the nanoparticles, problems with evaporation of volatile solvents during the measurements may be overcome. In the prior art method, great care must be taken when measuring the partitioning of a compound into a volatile solvent to avoid that the

solvent evaporates, changing the volume of the solvent, during the measurement making calculation of the partition coefficient complex. In the present method however, the volatile solvent may be absorbed into the porous outer coating of the nanoparticles which lowers the rate of evaporation of the solvent. Coupled with much faster partitioning of the test molecule, leading to overall lowering of the measurement time, this allows partitioning of a test molecule into a volatile solvent using the method of the present invention.

Fourthly, the present method does not rely on a visual determination of the solvent interface in order to achieve separation of the two solvents since separation is achieved by separation of the nanoparticles e.g. by magnetic separation, filtration or centrifugation. Hence this method of measuring the partition coefficient of a test molecule can be performed using much lower volumes of solvent than the prior art method. A lower volume of solvent lowers the cost of the measurement and facilitates automation of the process. Additionally, measurements using a lower volume of solvent produce less hazardous waste which further lowers both cost and environmental impact of the process.

Finally, the present process can be used to measure partition coefficients even in the case where a test molecule is highly soluble in one of the solvents. As mentioned above, the suitable ratio of first solvent to

second solvent is determined by the solubility of the test molecule in each solvent. In the method of the present invention, a wider range of first solvent to second solvent ratios may be used in the measurement of partition coefficient values. This is due to the fact that separation of the solvents prior to measurement of the concentration of the test molecule does not require a visual determination of the boundary between the first and second solvents. As such ratios of between 3000:1 to 1:1 first solvent:second solvent can be used. The use of small quantities of solvents, test molecule and composite nanoparticles coupled with the fast spectroscopic determination of test molecule concentration in supernatant allow a rapid evaluation of partition coefficient. Thus, a high throughput screening of a wide variety of test molecules in robot-friendly manner can be established.

Catalytic species encapsulated within a nanoparticle with a porous outer coating.

The advantages of nanoparticles within a porous outer coating encapsulating catalytically active species are wide ranging due to the variety of outer coatings and catalytically active species which can be envisaged. The use of the porous-coated nanoparticles of the present invention in heterogeneous inorganic catalysis is

envisaged, as is also their use in containing biologically active species.

In one aspect nanoparticles with a core containing a magnetic material are particularly useful as their catalytic activity can be exploited in suspension and separation of the catalyst from the product of the reaction is easily achieved using a magnetic field.

A further advantage of the nanoparticles of the present invention when the core material comprises a biologically active species, is that the biologically active species may not be chemically altered compared with its free state, for example by attachment of solubilising groups or linker groups to bind the species to a substrate. This means that the physical structure of the contained species is not altered by binding to pendant groups or the like. Thus in this aspect of the invention the species behaves in a similar way to the non-encapsulated form.

It is also known that a suspension of biologically active molecules, such as enzyme molecules, aggregates in solution if the concentration of the molecules is raised above a certain threshold. This limits the use of biologically active species at high concentration in suspension, since aggregation will lead to a drop in the surface area to volume ratio of the molecule and hence a potential lowering in the number of binding sites available to interacting molecules. In the present

invention however, the porous outer coating surrounding the biologically active species prevents aggregation of the molecules and allows the species to be present in suspension to higher concentration than with the prior art methods.

The broad applicability of the encapsulation method to various core materials and range of potential porous outer coatings results in a huge variety of potential applications for the nanoparticles of the present invention. Applications which are envisaged include assay methods for a variety of drug molecules, measurement of molecular binding constants, catalysis reactions (in both biological and chemical systems), biosensor applications, antibody-antigen, storage and release, etc. One example is encapsulation of albumin, for study of drug/albumin interaction and/or determination of binding constant.

Examples

Example 1 - Formation of porous-silica coated Fe_3O_4 nanoparticles.

Formation of a microemulsion was carried out using de-ionized water, excess pre-dried toluene and ionic surfactant (CTAB). Typically, the experiment was carried out at room temperature. The microemulsion was formed as follows: 0.02 mol CTAB (99%, Aldrich) was added into 100g dried toluene (99+%, Fisher) under vigorous stirring to

create a well-distributed suspension of CTAB in toluene. 0.3428 g $FeCl_2 \cdot 4H_2O$ and 0.9321 g $FeCl_3 \cdot 6H_2O$ (both 99%, Aldrich) were dissolved in 6.2 g water. This solution was added slowly in droplets into the toluene suspension of CTAB in nitrogen atmosphere. After stirring for 4 h, NH_3 solution (18.1 M, 1 ml, Fisher) was then added to the mixture. After an hour the whole system turned black in color. It has been previously shown that the addition of Fe^{2+}, Fe^{3+} and ammonia will produce magnetic Fe_3O_4 precipitate. At this point tetraethyl orthosilicate (TEOS) (99%, Aldrich) was added into the reaction mixture. Nitrogen was bubbled through the mixture for one hour. The ammonia solution (high pH) catalyzed hydrolysis/condensation of the TEOS into silica-gel. The silica over-layers were aged for 5 days in suspension. Finally, the precipitate was isolated by magnetic separation means and washed several times with hot ethanol, water and acetone to remove surfactant and organic solvents. The precipitate was then dried at room temperature resulting in a deep brown powder.

Example 2 - Analysis of the product of example 1.

The product obtained in example 1 was analysed using a variety of techniques.

The particles showed a strong magnetic response upon exposure to magnetic field showing a super-paramagnetic response(see figure number 3).

The Fe_3O_4 nanoparticles are shown by transmission electron microscopy (TEM) to be approximately 12 nm in diameter (see figure 4 whereas calculations from X-ray diffraction (XRD) measurements indicate that the particles are around 17 nm in diameter (see figure 5).

The chemical composition of the nanoparticles was measured by energy dispersive spectrometry (EDS) (see table 1)

Table 1. Energy Dispersive Spectrometry Analysis of the synthesized nanoparticles.

	Element Atomic (%)		
	Fe	O	Si
Site 1	24.21	60.28	15.51
Site 2	24.60	63.94	11.46
Site 3	23.87	62.46	13.67
Site 4	23.59	60.94	15.47
Site 5	24.84	61.50	13.66
Site 6	23.39	62.59	14.02
Average Value	24.08	61.95	13.97

Calculations from table 1 suggest that the composition of the silica-coated Fe_3O_4 individual particles is $Fe_3O_{4.24} \cdot 1.74SiO_2$. Further experiments forming particles

using the method of example 1 but varying the TEOS concentration suggest that nanoparticles with a composition of $Fe_3O_{4.1} \cdot 0.21SiO_2$ can also be obtained. This suggests that tailoring of the thickness of the silica coating on the nanoparticles is possible using the method of example 1.

Example 3 - Measurement of the porosity of the silica coating on the nanoparticles produced in example 1.

An estimation of the maximum amount of n-octanol which can be trapped in the pores of the silica coating of Fe_3O_4 nanoparticles produced by the method of example 1 can be obtained by thermogravimetric (TG) analysis (see figures 6 and 7). The values obtained from TG analysis of the product prepared by the method of example 1 suggests that the silica coating can absorb up to 0.54 ml per 1 g nanoparticles. BET (>300 m^2 per gm of silica) and pore size measurements (pore size range from 0.5-3 nm) also indicate that the composite nanoparticles are porous in nature.

Example 4 - Capping of surface hydroxyl groups

Porous-silica-coated Fe_3O_4 nanoparticles obtained by the method of example 1 were further modified to cap surface hydroxyl groups on the silica coating with trimethyl silyl ($-Si(CH_3)_3$) groups. Excess CTMS was allowed to flow through a fixed bed of dried silica-gel

coated Fe_3O_4 in nitrogen gas at 120°C. IR spectra of the porous-silica coated Fe_3O_4 nanoparticles before and after treatment with CTMS are shown in figure 8 showing the decrease in intensity of the Si-OH signal (~967 cm^{-1}) and appearance of the Si-CH_3 signal (~850 cm^{-1} and ~1265 cm^{-1}) which indicates the capping of the -OH groups on the silica surface.

Example 5 - Formation of porous-silica coated Fe_3O_4.

Fe_2CoO_4 nanoparticles were produced and coated with a porous silica coating by the same method as in example 1. In this case, equal molar amounts of $FeCl_3.6H_2O$ (the same amount used as described in Example 1) and $CoCl_2.xH_2O$ were dissolved in water which was added to the toluene suspension of CTAB in the same manner as example 1. The size of the Fe_2CoO_4 particles was measured by XRD (figure 9) as approximately the same size as the Fe_3O_4 particles formed in example 1.

Example 6 - Measurement of logD values using porous-silica coated nanoparticles.

Potassium dihydrogen orthophosphate (99%, Aldrich) 0.1 mM aqueous solution, pH value adjusted to be 7.4, was used as a buffer solution in the following measurements. The test molecule was dissolved into the buffer solution that had already been pre-saturated with *n*-octanol in a glass vial. The test molecule concentration was kept at

about 1×10^{-5} M. *n*-octanol, 10 to 100 μl, pre-saturated with the buffer solution was physically absorbed onto the porous-silica coated Fe_3O_4 nanoparticles (obtained by the method of example 1) by capillary action. The nanoparticles were allowed to disperse into a known concentration of the test molecule solution. The volume ratio of aqueous solution to *n*-octanol in the mixture was set at 100 : 1. The glass vial was sealed and put in an orbital shaker. The shaking speed was carefully controlled to avoid any *n*-octanol droplets detaching from the composites (visually). After shaking an external magnet was placed near the bottom of the vial. Magnetic induced precipitation was achieved in a few minutes or less. UV-visible absorptions of the test molecule analyte in the supernatant aqueous phase before and after precipitation were measured with baseline correction. The logD value was then obtained using the equation below:

$$\log D = \log \{[(A1-A2)/A2]\times V_w/V_o\}$$

where: A1 = UV-visible absorption of the test molecule in the supernatant phase before partitioning.

A2 = UV-visible absorption of the test molecule in the supernatant phase after partitioning.

V_o = Volume of n-octanol (absorbed into the porous outer coating of the nanoparticles).

V_w = Volume of water (with which the nanoparticles are mixed).

Unless otherwise stated the volume ratio of the first solvent (water) to the second solvent (n-octanol) was fixed to be 100:1.

Partition coefficient values of some test molecule analytes were measured with the surface -OH groups of the nanoparticles capped with trimethyl silyl (TMS) groups to compare with un-capped nanoparticles.

For comparative purposes the partition coefficient of each test molecule analyte was independently measured by a prior art "shake-flask" method with the same test molecule concentration and the phase volume ratio. The results of these measurements are shown in table 2 below.

Table 2

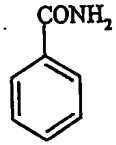
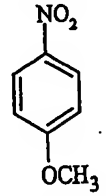
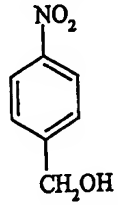
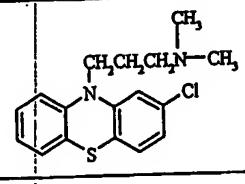
Molecule	Structure	LogD value from literature	LogD value measured (Prior art method) (pH=7.4)	Mean logD value measured (Prior art method) (pH=7.4)	LogD value measured by present invention (pH=7.4)	Mean logD value measured by present invention (pH=7.4)
Benzamide		0.660	0.643	0.654±0.0094	0.639	0.678±0.0460
			0.651		0.656	
			0.663		0.704	
			0.654		0.719	
			0.658		0.673	
4-Nitroanisole		2.030	2.020	2.010±0.0077	2.014	2.013±0.0120
			2.006		2.023	
			2.011		2.020	
			2.004		2.002	
			2.010		2.008	
4-Nitrobenzyl alcohol		1.260	1.252	1.257±0.0119	1.266	1.268±0.0381
			1.264		1.295	
			1.244		1.234	
			1.256		1.296	
			1.268		1.250	
Chlorpromazine		3.200	3.075	3.076±0.0047	3.144	3.164±0.0111
			3.074		3.179	
			3.076		3.186	
			3.083		3.124	
			3.074		3.186	

Table 2 (continued)

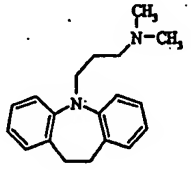
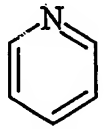
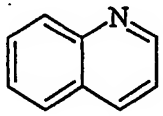
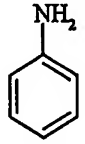
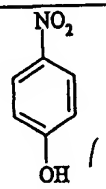
Molecule	Structure	LogD value from literature	LogD value measured (Prior art method) (pH=7.4)	Mean logD value measured (Prior art method) (pH=7.4)	LogD value measured by present invention (pH=7.4)	Mean logD value measured by present invention (pH=7.4)
Imipramine		2.500	2.511	2.561±0.0348	2.686	2.589±0.1632
			2.579		2.445	
			2.573		2.678	
			2.569		2.691	
			2.571		2.446	
Pyridine		0.650	0.674	0.681±0.0221	0.693	0.696±0.0171
			0.699		0.687	
			0.654		0.696	
			0.691		0.702	
			0.689		0.704	
Quinoline		2.020	2.116	2.116±0.0039	2.191	2.186±0.0044
			2.115		2.185	
			2.113		2.182	
			2.121		2.184	
			2.114		2.188	
Aniline		0.900	0.936	0.934±0.0094	0.963	0.973±0.0303
			0.945		0.990	
			0.929		0.935	
			0.926		0.990	
			0.932		0.989	

Table 2 (continued)

Molecule	Structure	LogD value from literature	LogD value measured (Prior art method) (pH=7.4)	Mean logD value measured (Prior art method) (pH=7.4)	LogD value measured by present invention (pH=7.4)	Mean logD value measured by present invention (pH=7.4)
4-Nitrophenol		1.480	1.771	1.785±0.0190	1.814	1.807±0.0052
			1.779		1.803	
			1.811		1.808	
			1.785		1.806	
			1.780		1.805	

Example 7 - Formation of nanoparticles with an enzyme core

A first buffer solution was prepared comprising potassium dihydrogenphosphate 0.01 mol and sodium chloride 0.25 mol in 500 ml de-ionized water. The buffer pH value was adjusted to 7.0 by addition of sodium hydroxide solution at 20°C.

Penicillinase (β-Lactamase I, purified from Bacillus cereus, Sigma) was then dissolved in a second buffer solution of the same composition to an enzyme concentration of 50 nM. A micro-emulsion was formed as in example 1 (0.02 mol CTAB in 100 g dried toluene) to which 5.2 g of the first buffer solution was added slowly in droplets with continuous stirring. 2 ml of the second buffer solution (containing penicillinase) was added over 4 hours. The mixture was then stirred for a further four hours to ensure an even dispersion of enzyme molecules in the micro-emulsion system. A 200 µl sample of the mixture was removed for comparison of enzymatic activity (see example 8). 6.94 g TEOS was then slowly added to the system, which underwent hydrolysis at the water/toluene interface to form the external silica coating.

Example 8 - Enzymatic activity test on the product of example 7.

The 200 µl sample of the mixture removed prior to addition of TEOS in example 7 was analysed, using UV-visible spectroscopy to follow the hydrolysis of the lactam group of

pencillin at 232 nm, to determine whether the enzyme is still functional through hydrolysis of a calibrated standard penicillin V (Phenoxymthylpenicillinic acid) (3 nM, Sigma). A further 200 µl sample of the mixture from example 7 was extracted six days after addition of the TEOS and analysed in the same manner.

UV-visible spectra are shown in figures 10 to 12. The spectral curves represent principally the UV-visible spectra of the penicillin V. Hydrolysis of Penicillin V by the added free form of β-Lactamase I showing a typical UV-visible spectral change is plotted in figure 10. It can be clearly seen that the absorbance value at 232 nm (the region where lactam group absorbs) decreases over five minutes indicating that rapid conversion of the penicillin V to corresponding penicilloic acid is occurring (hydrolysis of the lactam group). Figure 11 shows the result of penicillin V solution upon the addition of the 200 µl sample extracted from the reaction mixture before TEOS addition (no silica coat). The absorbance value at 232 nm where the lactam group is located is again attenuated over the 5 minute period shown. This indicates that the enzyme remains active when incorporated into micelles of the mixture. Figure 12 also shows that a similar spectral change is observed when using the sample extracted six days after TEOS addition to the mixture of example 7 (silica coated nanoparticles). The absorbance value

at 232 nm clearly continued to decrease over the five minutes between the two spectra.

These results indicate that the entrapped enzyme remains functional in both the micelle and the silica-coated composite environments.

CLAIMS

1. A composition comprising nanoparticles having a porous surface and a first solvent, wherein a second solvent is absorbed into the pores of the nanoparticles and wherein said first and second solvents are immiscible.

2. A composition according to claim 1 wherein said nanoparticles form a colloidally stable suspension in said first solvent.

3. A composition according to claim 1 or 2 wherein said porous surface is formed OF any one of silica, alumina, titania, zirconia or carbon.

4. A composition according to any one of the preceding claims wherein the nanoparticles further comprise a magnetic material core.

5. A composition according to claim 4 wherein said magnetic material core is formed from magnetite (Fe_3O_4), maghemite (γFe_3O_4), greigite (Fe_3S_4), Fe_2CoO_4, a ferromagnetic metal or alloy or carbide.

6. A composition according to any one of the preceding claims wherein said nanoparticles have a diameter of between 2nm and 1μm.

7. A composition according to any one of the preceding claims wherein the porous surface layer of said nanoparticles has a thickness of between 1nm and 100nm.

8. A composition according to any one of the preceding claims wherein said first solvent is aqueous.

9. A composition according to claim 8 wherein said aqueous solvent is water.

10. A composition according to any one of the preceding claims wherein said second solvent is one of n-octanol, cyclohexane, a C_6 - C_{10} alkane, chloroform, propylene glycol dipelargonate (PGDP), 1,2-dichloroethane, olive oil, benzene, toluene, nitrobenzene, chlorobenzene, tetrachloromethane, oleyl alcohol, 4-methylpentan-2-ol, pentan-1-ol, pentan-2-ol, isobutanol, butan-1-ol, 2-methylbutan-2-ol, butan-2-ol, butan-2-one, diethyl ether, isoamyl acetate, ethyl acetate, etc. or a monophasic mixture of two or more of these.

11. A composition according to any one of the preceding claims wherein the volume ratio of said first solvent to said second solvent is between 3000:1 and 1:1 (preferably in the range 500:1 to 50:1).

12. A composition according to claim 11 wherein the ratio of said first solvent to said second solvent is at least 100:1.

13. A method of attaining partition of a compound between two immiscible solvents comprising incorporating said compound in a composition according to any one of the preceding claims.

14. A composition for use in a quantitative analytical technique, comprising nanoparticles each having a porous surface and a solvent adsorbed in the pores of the nanoparticles in a predetermined amount per unit weight of the composition.

15. A composition according to claim 13, wherein said porous surface is formed from any one of silica, alumina, titania, zirconia or carbon.

16. A composition according to claim 14 or 15 wherein the nanoparticles each have a magnetic material core.

17. A composition according to any one of claims 14 to 16 wherein said solvent is immiscible with water.

18. A composition according to claim 17 wherein said second solvent is one of n-octanol, cyclohexane, a C_6 - C_{10} alkane, chloroform, propylene glycol dipelargonate (PGDP), 1,2-dichloroethane, olive oil, benzene, toluene, nitrobenzene, chlorobenzene, tetrachloromethane, oleyl alcohol, 4-methylpentan-2-ol, pentan-1-ol, pentan-2-ol, isobutanol, butan-1-ol, 2-methylbutan-2-ol, butan-2-ol, butan-2-one, diethyl ether, isoamyl acetate, ethyl acetate, etc. or a monophasic mixture of two or more of these.

19. Use of a composition according to any one of claims 14 to 18 in a method of determining a partition coefficient.

20. A method of measuring the partition coefficient of a compound between two immiscible solvents, said method comprising the steps of:

a) incorporating said compound in a composition according to any one of claims 1 to 13;

b) separating the product of step a) into two components, the first comprising the nanoparticles and the second comprising the first solvent; and

c) determining the partition coefficient from the partition of the compound between said first and second components.

21. A method according to claim 20 wherein step c) comprises determining the amount of said compound to be tested which remains in said first solvent.

22. A method according to claim 20 or 21 wherein said compound to be tested is a bioactive drug molecule.

23. A method according to any one of claims 20 to 22 wherein step b) is performed by any one of filtration, centrifugation ~~or~~ and magnetic separation.

24. A method according to any one of claims 20 to 23 wherein step c) comprises recording the UV-visible spectrum of said supernatant solution.

25. A method according to any one of claims 20 to 24 further comprising shaking the product of step a) prior to performing the separation step b).

26. A nanoparticle having a core comprising a catalytically active species, and a porous layer surrounding the core which

has a pore size such that the catalytically active species is entrapped.

27. A nanoparticle according to claim 26 wherein said core catalytically active species is a biologically active species, e.g. an enzyme or other protein.

28. A nanoparticle according to claim 27 wherein said biologically active species is any one of blood serum albumin, β-Lactamase I (Penicillinase),kinase, a carboxylesterase, metallothionin, cytochrome b, c, P450, etc.

29. A nanoparticle according to any one of claims 26 to 28 wherein said porous layer is formed from any one of silica, alumina, titania, zirconia or carbon.

30. A nanoparticle according to any one of claims 26 to 29 wherein said core further comprises a magnetic material.

31. A nanoparticle according to claim 30 wherein said magnetic core is formed from magnetite (Fe_3O_4), maghemite (γFe_3O_4), greigite (Fe_3S_4) or Fe_2CoO_4 or ferromagnetic metal or alloys (such as Fe-Pt, Fe-Co, Fe-Ni), metal carbides, etc.

32. A nanoparticle according to any one of claims 26 to 31 wherein said nanoparticles have an average a diameter of between 2nm and 1μm.

33. A nanoparticle according to any one of claims 26 to 32 wherein the core of the nanoparticle has an average diameter of between 1 and 10 nm.

34. A nanoparticle according to any one of claims 26 to 33 wherein the porous outer coating on said nanoparticle has a thickness between 1nm and 100nm.

35. An assembly of nanoparticles at least some of which are nanoparticles according to any one of claims 26 to 34, wherein on average the number of molecules of said catalyticaly active species per nanoparticle of the assembly is not more than one.

36. A method of making a nanoparticle according to any one of claims 26 to 34, comprising the following steps:

a) forming, in a liquid medium, colloidal particles containing the catalytically active species to be contained in the nanoparticle core, the particles being colloidally stabilised by a surfactant;

b) treating said colloidal particles by hydrolysis or pyrolysis to form the porous layer surrounding the catalytically active species.

37. A method of claim 36 wherein, in step a), said colloidal particles further contain a magnetic material or a precursor to a magnetic material.

38. A method of claim 36 or 37 wherein said colloidal particles comprise aqueous colloidal particles in a solvent which is immiscible with water.

39. A method of claim 38 further comprising adding a salt of silicon, aluminium, titanium or zirconium to the product of step a), which forms the corresponding oxide compound upon hydrolysis at the colloid boundary.

40. A method of claim 39 wherein said silicon salt is tetraethyl orthosilicate (TEOS) and the surfactant is cetyltrimethylammonium bromide (CTAB).

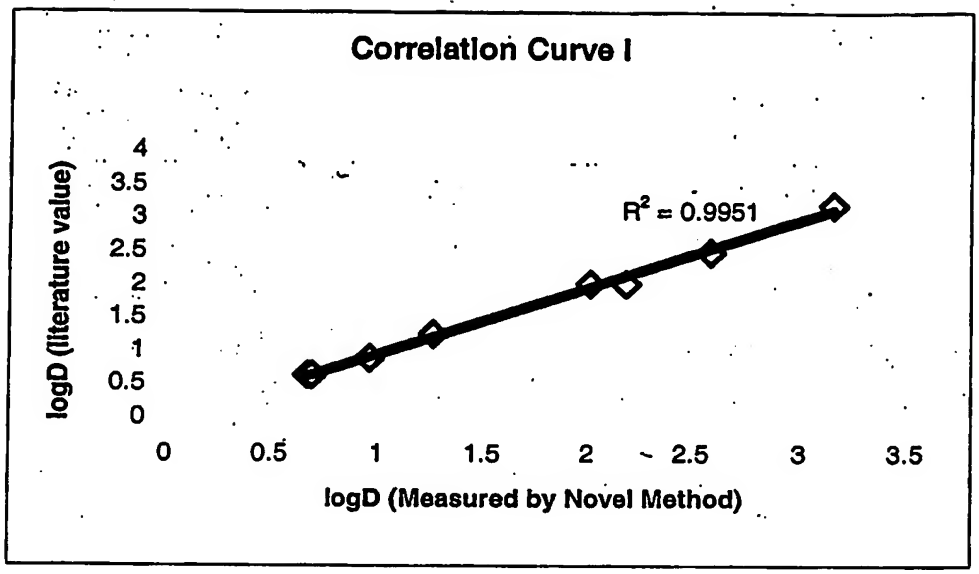

Correlation Curve I
logD (literature value)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
3.5
R² = 0.9951
logD (Measured by Novel Method)

Fig. 1

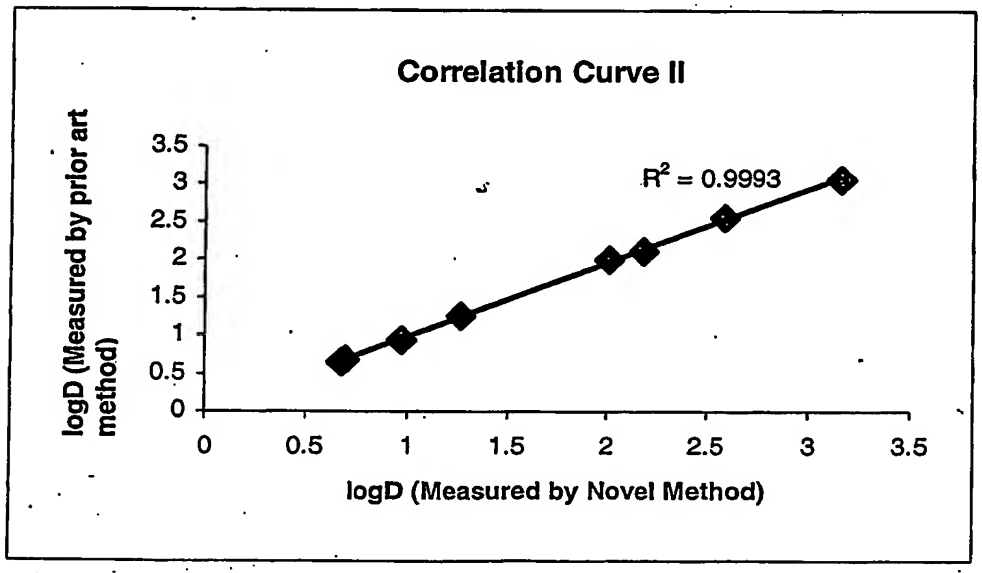

Correlation Curve II
logD (Measured by prior art method)
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
3.5
R² = 0.9993
logD (Measured by Novel Method)

Fig. 2

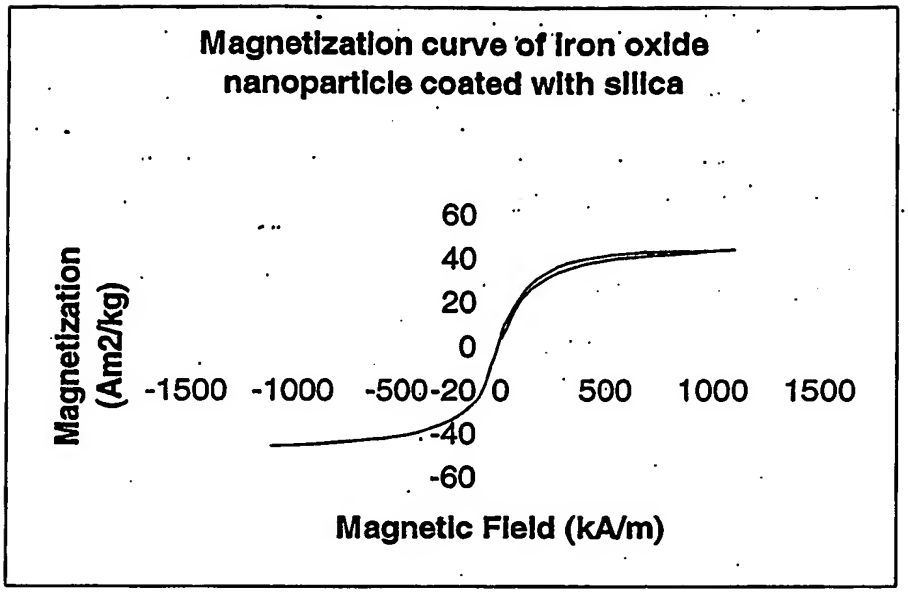

Magnetization curve of iron oxide nanoparticle coated with silica
Magnetization (Am2/kg)
60
40
20
0
-20
-40
-60
-1500
-1000
-500
0
500
1000
1500
Magnetic Field (kA/m)

Fig. 3

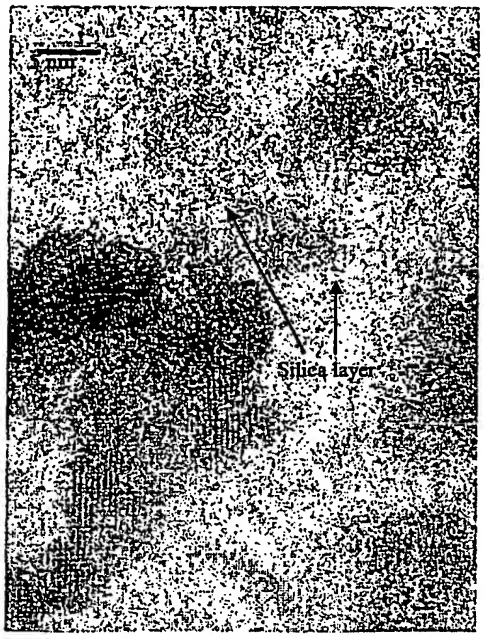
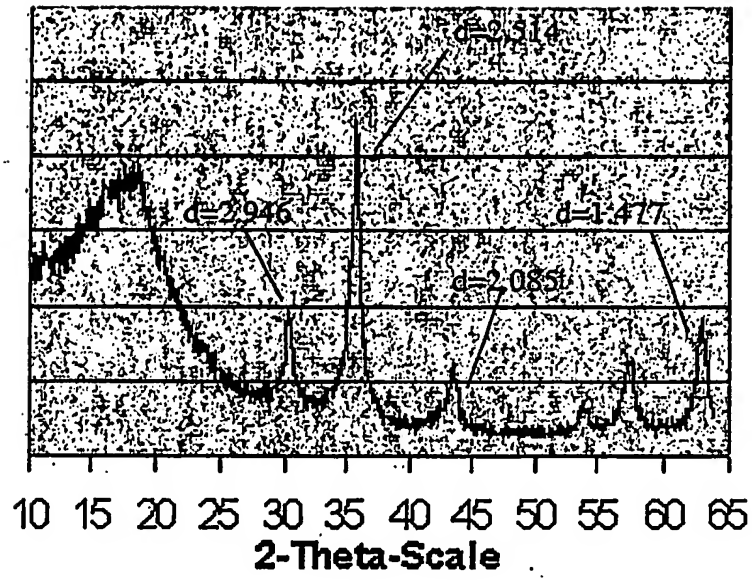

Silica layer

Fig. 4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d=2.946
d=1.477
d=2.085
10 15 20 25 30 35 40 45 50 55 60 65
2-Theta-Scale

Fig. 5

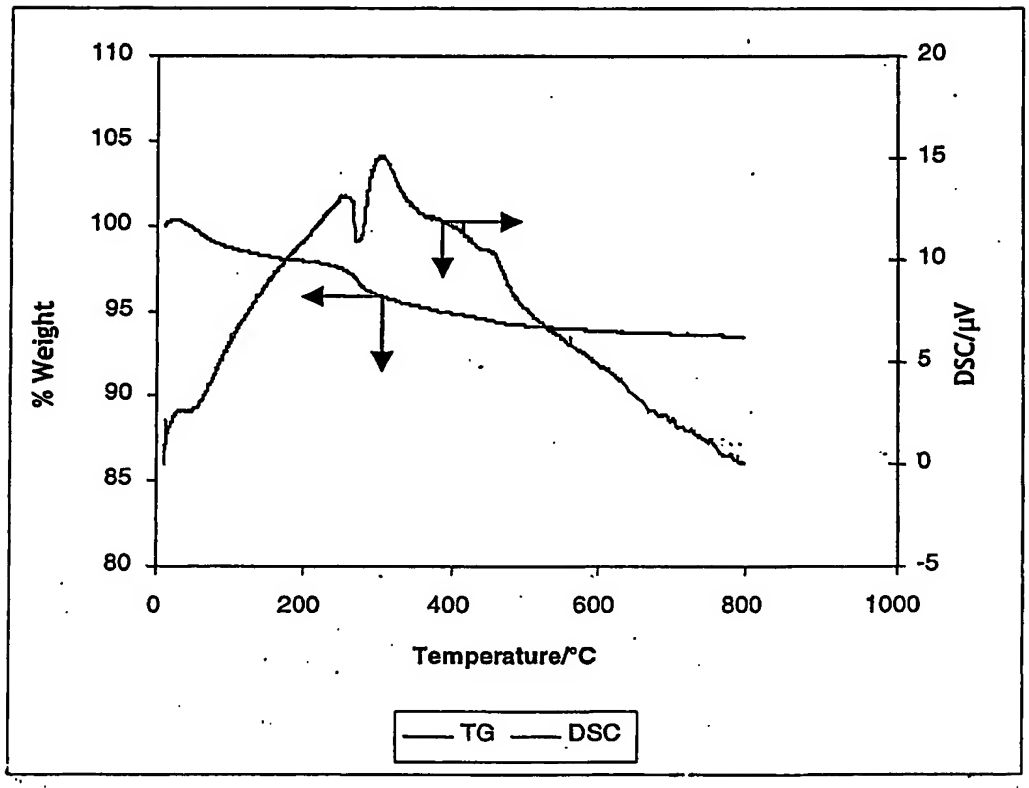
110
105
100
95
90
85
80
% Weight
20
15
10
5
0
-5
DSC/µV
0
200
400
600
800
1000
Temperature/°C
TG
DSC

Fig. 6

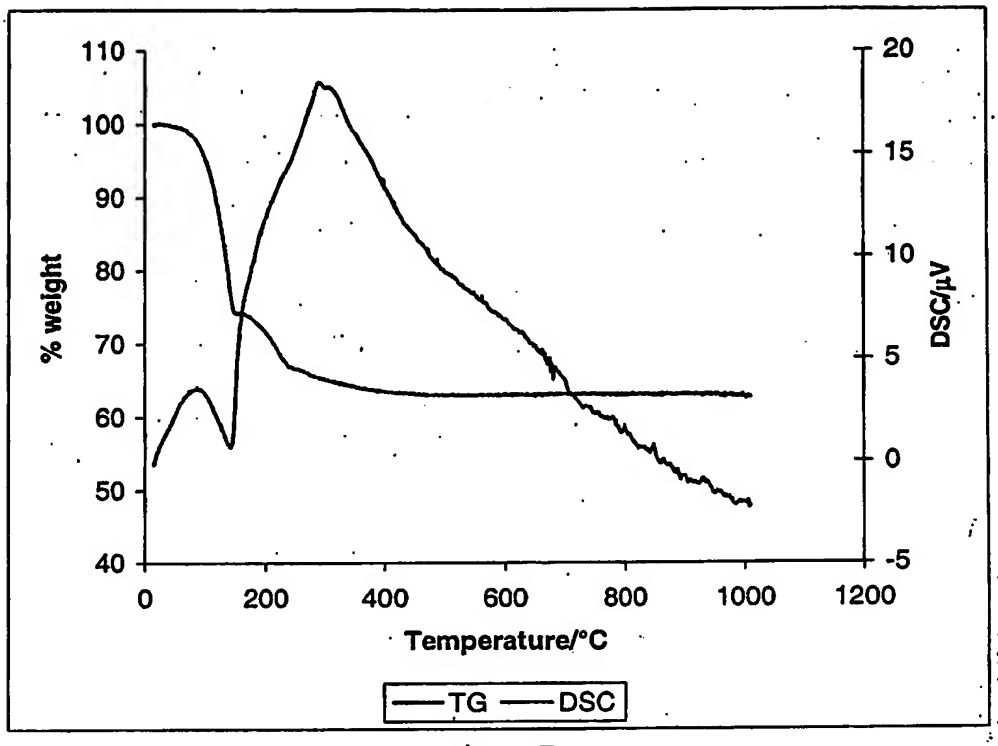
110
100
90
80
70
60
50
40
% weight
20
15
10
5
0
-5
DSC/µV
0
200
400
600
800
1000
1200
Temperature/°C
TG
DSC

Fig. 7

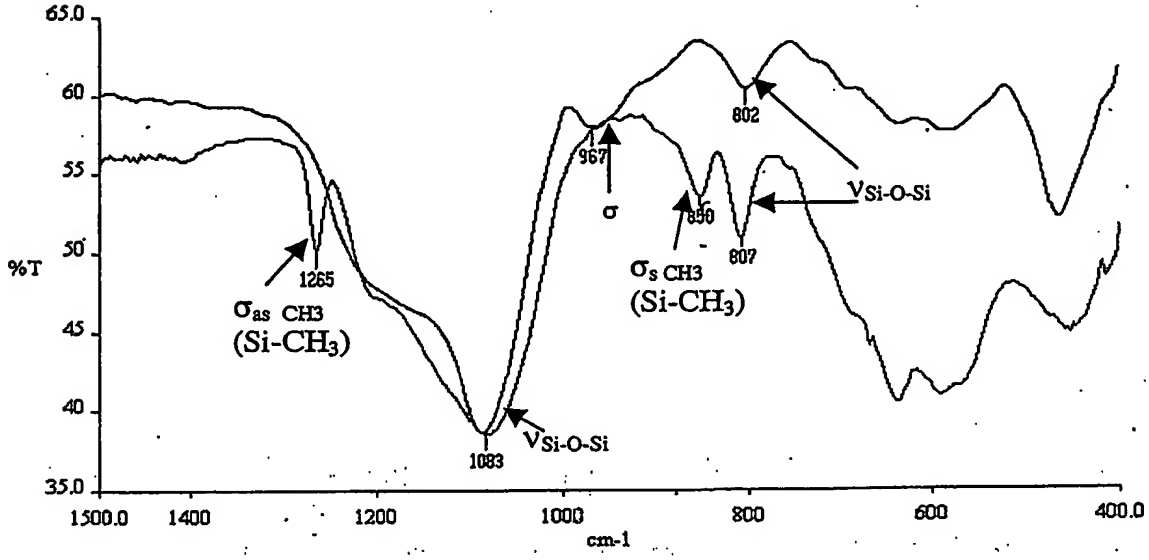
65.0
60
55
50
45
40
35.0
%T
1500.0
1400
1200
1000
800
600
400.0
cm-1
1265
σas CH3
(Si-CH3)
1083
νSi-O-Si
967
σ
890
807
σs CH3
(Si-CH3)
802
νSi-O-Si

Fig. 8

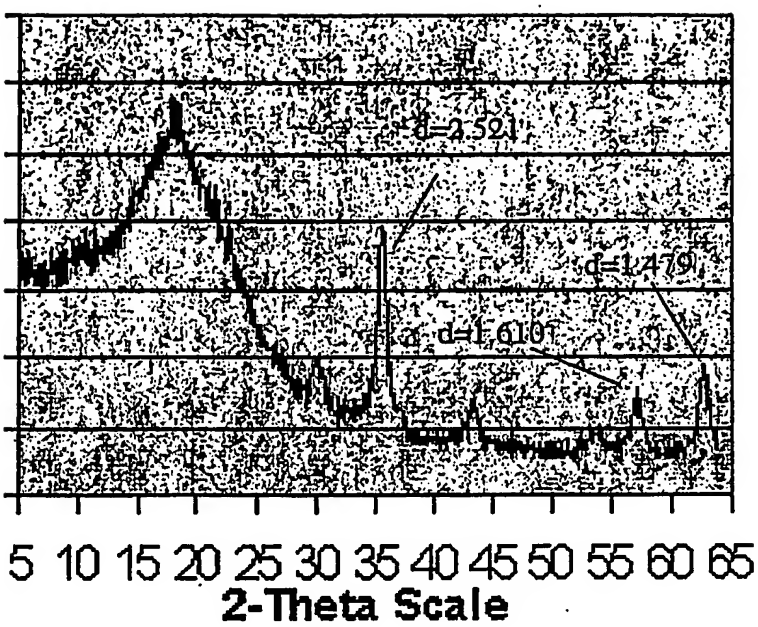
d=2.521
d=1.479
d=1.610
5 10 15 20 25 30 35 40 45 50 55 60 65
2-Theta Scale

Fig. 9

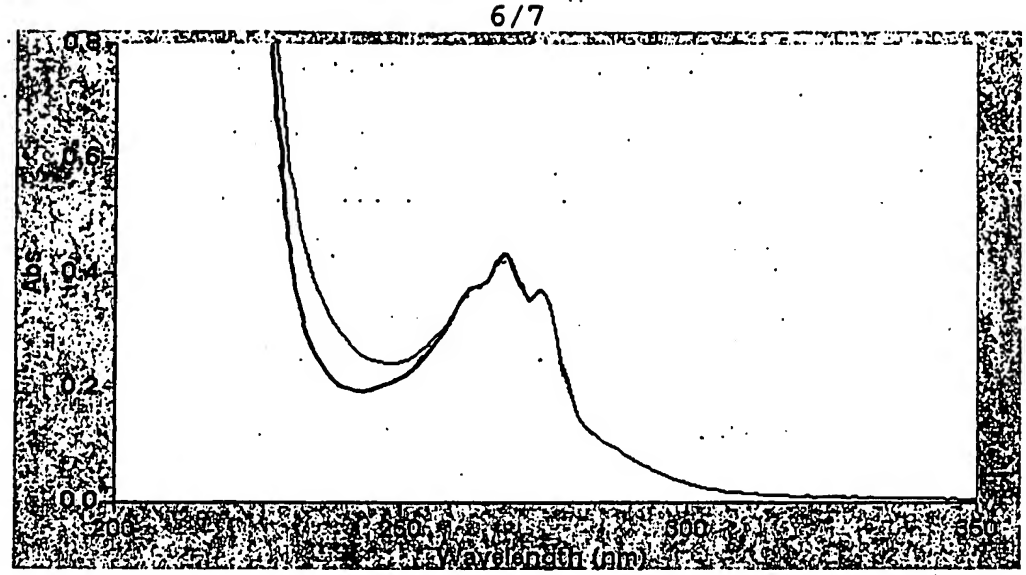
0.8
0.6
0.4
0.2
0.0
Abs
200
250
300
350
Wavelength (nm)

Fig. 10

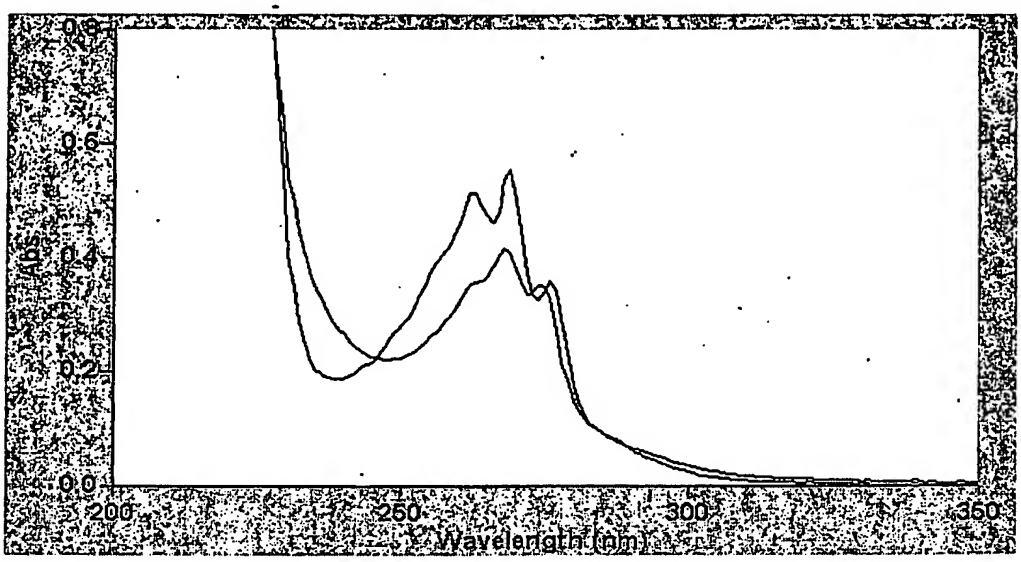
0.8
0.6
0.4
0.2
0.0
Abs
200
250
300
350
Wavelength (nm)

Fig. 11

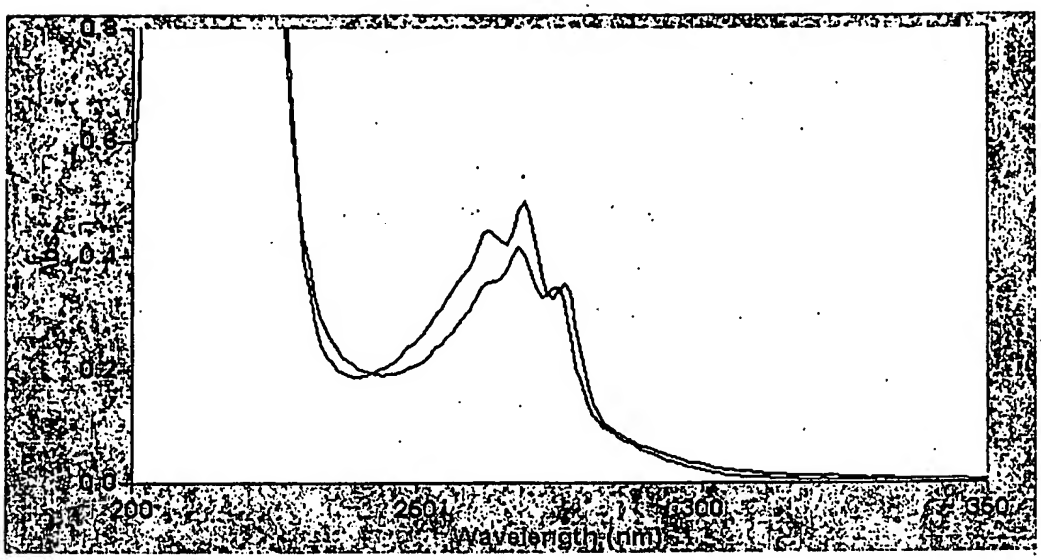

Fig. 12